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(54) Title: A PROCESS AND AN AGENT FOR DETECTION OF GENE STRUCTURES IN HUMANS HAVING A GREAT TENDENCY TO DEVELOP IDDM

(57) Abstract

An agent for detection of gene structures which are characteristic of humans having a tendency to develop insulin-dependent diabetes mellitus (IDDM), consisting of or containing a DNA sequence from the HLA-DG β-chain gene with 154 bp from intron 1 (IVS1) and adjoining first 12 bp of exon 2 or a reactive fragment thereof. There is moreover disclosed a process for identification of said DNA structure by hybridization of chromosomes from the individual to be tested with the agent in a labelled stated and detection of the labelled hybrid.

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A process and an agent for detection of gene structures in humans having a great tendency to develop IDDM

A process and an agent for detection of gene structures in humans having a great tendency to develop IDDM.

Insulin-dependent Diabetes mellitus, also called IDDM, is a disease which is often developed in an early age. IDDM is thus the most common chronic metabolic disease in children. The etiology has not been fully determined as yet, but it is assumed that the disease can be released by actuation of a still unknown virus or by other external effects.

The pathogenesis comprises phenomena of an autoimmune nature, including the presence of insulitis islet cell autoantibodies or organospecific autoantibodies or diseases of an autoimmune nature, cf. Gepts, W. "Pathologic anatomy and the pancreas in juvenile diabetes mellitus". Diabetes 14:619-633 (1965). Foulis A. K. and J. A. Stewart. "The pancreas in recent-onset Type 1 (insulin-dependent) diabetes mellitus: insulin cintent of islets, insulitis and associated changes in exocrine acinar tissue". Diabetologia. 26:456-461 (1984).

Individuals having certain hereditary characteristics have a particularly great tendency to develop IDDM. Although the hereditary factor has not been fully explained, it has been found that 95% of IDDM patients has antigens for the gene structure HLA-DR 3 and/or 4; Platz. P., B. K. Jakobsen, M. Morling, L. P. Ryder, A. Svejgaard, M. Thomsen, M. Christy, H. Kromann, J. Benn, J. Nerup and A. Green, "A genetic analysis of insulin-dependent diabetes mellitus, Diabetologia 21, 108-115 (1982), and Wolf, E., K. M. Spencer and A. G. Cudworth "The genetic susceptibility to type I (insulin-dependent) diabetes. "Analysis of the HLA-DR association", Diabetologia 24, 224-230 (1983). HLA-DR 3/4 heterozygous individuals thus have the greatest

risk of developing IDDM.

Though almost all IDDM patients have antigens for HLA-DR 3 and/or 4, determination of this gene is not suitable for identification of individuals having a great tendency to develop IDDM, since the frequency of individuals having such specific hereditary characteristics is about 60% of the entire population, and just a small fraction actually develops IDDM. Even in families with two or more children having IDDM, the risk of an HLA-DR identical sister or brother is only 15-30%, cf. the above-mentioned literature.

It is known to use an HLA-DQ β-chain cDNA probe, Böhme, J., B. Carlsson, J. Wallin, E. Möller, B. Persson, P. A. Peterson and L. Rask. "Only one DQ β restriction fragment pattern of each DR specificity is associated with insulin-dependent diabetes", J. Immunol., 1986, for detection of gene structures characteristic of individuals having a great tendency to develop IDDM. Such a probe can be used for tissue type determination of individuals having a great tendency to develop IDDM since the probe hybridizes to HLA-DQ β-chain genes.

The probe in question is produced from genomic DNA from lymphocytes by cleavage with BamHI restriction enzyme and isolation of a 3.7 kb fragment.

It is also known that the HLA-DQ β-chain probe is non-specific.

The present invention is based on the finding that a considerably smaller DNA sequence, which forms part of the mentioned 3.7 kb fragment, has increased specificity and is particularly useful as a probe for tissue type determinations to identify individuals having a risk great of developing IDDM. This DNA sequence, called IVSI (166

bp), and fragments of it have not been produced or isolated before.

Thus, the invention concerns an agent for detection of gene structures which are characteristic of individuals having a tendency to develop insulin-dependent diabetes mellitus (IDDM), consisting of or containing a DNA sequence from the HLA-DQ β-chain gene, and the agent is characterized in that the DNA sequence comprises 154 bp from intron 1 (IVSI) and the adjoining first 12 bp of exon 2 or a reactive fragment thereof.

The invention also concerns a process for producing the agent, said process being characterized by hybridizing chromosomes from a human cell with the agent of claim 1 or 2 in a labelled state, and then detecting the hybrid thus labelled.

The present probe, IVSI (166 bp), hybridizes with the HLA-DQ β-chain gene, which can be detected by known techniques for tissue type determination.

In the process of the invention for identification of individuals preferably having a great tendency to develop IDDM, DNA is isolated from nucleated blood cells from the individual in question, and they are hybridized with an IVSI (166 bp) probe. Hybridization is usually effected by usual blotting technique, using labelling of the IVSI probe with radioactive isotopes for detection of positive reaction.

The invention will be illustrated more fully below by means of a working example.

EXAMPLE 1

The procedure started with a Swedish family without IDDM

and without NIDDM for cloning of a BamHI 3.6 kb fragment from an HLA-DR 4 containing chromosome, cf. Michelsen, B., W. Kastern, Å. Lernmark, and D. Owerbach "Identification of and HLA-DC β-chain related genomic sequence associated with insulin-dependent diabetes". Biomed. Biochem. Acta, 44:33-36 (1985). The individual giving blood for cloning was a healthy HLA-DR2/4, 3.7 kb fragment positive mother with two daughters. Her husband is HLA-DR3/7, and their two daughters are HLA-DR2/3 and 4/7, respectively. The HLA-DR4/7 daughter also has the BamHI 3.7 kb fragment, which is therefore present on the HLA-DR4 containing chromosome in the mother. Mononuclear cells were obtained from 30 ml of blood by Ficoll-Hypaque gradient centrifugation, and DNA was extracted as described below. About 50 µg of DNA were digested with BamHI and electrophoresed in 1% agarose gel together with suitable molecular weight markers. The region 3.4 to 3.8 kb was cut off from the gel, and the DNA fragments were recovered by electroelution. This fraction of the fragment was ligated into the BamHI site in pUC8 and used for transforming E. coli JM 10528. Recombinant plasmids were identified as those giving white colonies on LB plates with 20 µg/ml of both IPTG and BCIG, and they were screened for HLA-DQ related sequences by in situ hybridization on nitro cellulose filters with nick translated DQ β-cDNA probe, cf. the above-mentioned literature.

Production of DNA

Mononuclear cells, obtained from 10 ml blood by Ficoll-Hypaque gradient centrifugation, were digested overnight at 37 °C in 0.02% proteinase K and 1% weight/volume of sodium dodecyl sulfate (SDS) in 10 mM Trsi-HCl to buffer (pH 7.4) containing 1 mM EDTA (TE buffer). After phenol and chloroform extractions, DNA was precipitated with ethanol and resuspended in TE buffer.

Genomic blots

10 to 20 µg of lymphocyte DNA were digested with the restriction enzyme BamHI (Boehringer Mannheim, DE), electrophoresed overnight at 40 V in 1% flat agarose gels and then transferred to "HybondN" nylon membranes (Amersham, Buckingshire, GB) in a known manner.

Hybridizations

Filters having a DQβ-cDNA probe were prehybridized for 2 to 16 hours in 50% formamide, S x SSC, 5 x Denhart's solution, 50 mM Na_2PO_4 (ph 6.5) and 0.5 mg/ml denatured salmon sperm DNA. Hybridization was performed overnight in 50% formamide, 1 x Denhart's solution, 20 mM Na_2PO_4 (pH 6.5), 10% dextran sulfate, 0.2 mg/ml denatured salmon sperm DNA and 10^6 dpm/ml denatured probe. The washing stringency was 0.1 x SSC at 55 °C.

Probes

The DQβ cDNA probe was derived from the plasmid pII-β1, cf. Larhammer, D., L. Schenning, K. Gustafsson, K. Wiman, L. Claesson, L. Rask, and P. A. Peterson, "Complete amino acid sequence of an HLR-DR antigenlinker β chain as predicted from the nucleotide sequence: Similarities with immunoglobins and HLA-A, -B, and -C antigens." Proc. Natl. Acad. Sci. USA 79:3687-3691 (1982), by digestion with PstI and EcoRI, and then the 800 bp fragment was eluted from agarose melting at a low temperature ("BioRad", CA).

A subclone of the BamHI 3.7 kb fragment cloned in pBR322 was used for constructing the IVSI probe. The recombinant plasmid was digested with RSA1 to provide a fragment which contains 154 base pairs from the intron and 12 base pairs from the other exon, which codes for the first region of the HLA-DQ β-chain as well as the part of pBR322 dis-

posed from the BamHI site in position 375 to the RSAI site in position 2281. This 2072 base pair fragment was purified by agarose gel electrophoresis in low melting agarose.

Labelling of probes was done by nick translation using "α-32P-dCTP" (Amersham) at 30 TBq/mmol (DQβ probe) or 110 TBq/mmol (IVSI probe).

Construction of a series of progressive deletions for sequence formation

The BamHI 3.7 kb genomic insert was subcloned in the BamHI site by pUC19, cf. Yanisk-Perron C., J. Vieira and J. Messing "Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors". Gene 33: 103-119 (1985). This structure was then linearized with the restriction enzymes SphI and HindII. SphI digestion between the BamHI site in pUC19 and the associated site for the reverse sequence primer (Amersham) was performed and gave a 4 base 3'-overhang, which cannot be obtained with "Exonuclease III", (Pharmacia PL Biochemicals, Uppsala, SE), cf. Henikoff, S. "Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing". Gene 28:351-359 (1984). HindII digestion with the polylinker sequence from pUC19 between the SphI site and the BamHI cloning site was used for forming a sticky end which is susceptible to exonuclease III. There were no sites for Sphl and HindIII in the insert. Unidirectional deletions disposed from the primer associated site in pUC19 to various points in the inserted fragment were isolated by digestion of plasmid DNA with Sphl and HindII followed by phenol/chloroform extraction and ethanol precipitation. The linearized plasmid DNA was resuspended to 0.1 g/litre in 6.6 mM Tris-HCl (pH 7.4) containing 6.6 mM $MgCl_2$ and was incubated at 37 °C for 5 minutes before addition of exonuclease III to a final concentration of 10 units/

/ulitre. At intervals of 15 seconds, 35 aliquots of 10 /ulitres were transferred to 30 /ulitres 0.2 M NaCl containing 5 mM EDTA and heated to 70 °C for 10 minutes to inactivate the enzyme. DNA was precipitated by addition of 120 /ulitres of ethanol, centrifuged, and each pellet was resuspended in 80 /ulitres of 0.05 M NaOAc (pH 4.5) containing 0.1 M NaCl, 30 mM $ZnSO_4$ and 75 Vogt units/ml S1 nuclease (Boehringer) for removal by incubation for 30 minutes at room temperature of the singlestranded 3'- and 5'-end projections formed by SphI and exonuclease III, respectively. The reaction was terminated with phenol/chloroform extraction followed by ethanol precipitation. Pellets were resuspended in 10 /ulitres of 25 mM Tris HCl (pH 7.4) containing 5 mM $MgCl_2$, 5 mM dithiothreitol (DDT), 0.25 mM spermidine, 1 mM ATP, 10 /ug/ml BSA and 700 units/ml of T4 DNA ligase (Amersham) and ligated at room temperature overnight. E. coli KM109 was transformed with 5 /ulitres of each of the ligated fractions according to Hanahan, cf. Hanahan D. "Studies on transformation of Escherichia Coli with plasmids". J. Mol. Biol., 166: 557-580 (1983), the bacteria cells were scattered on LB plates containing 50 /ug/ml of ampicillin. About 10 to 20 transformants from each aliquot were selected for characterization of the deletion size by agarose gel electrophoresis. Each clone was cultivated overnight in a 5 ml L bouillon culture in the presence of 50 /ug/ml ampicillin. Plasmid preparations were produced on a small scale by the basic lysis method, cf. Maniatis, T., E. F. Fritsch and J. Sambrook. "Molecular Cloning - a laboratory manual", Cold Spring Harbor Laboratory (1982), with the following modifications. After precipitation with potassium acetate, the supernants were centrifuged for 2 minutes, transferred to new tubes containing 0.6 volume isopropanol and centrifuged for 5 minutes at 4 °C. Pellets were washed with 80% ethanol and resuspended in TE. A volume 4 M LiCl of the same size was added. After 5 minutes at 0 °C, the precipitates were collected by centrifugation for 2 mi-

nutes. The supernatants were incubated for 30 minutes at 37 °C with 25 µg/ml RNase A followed by phenol/chloroform extraction. Plasmid DNA was then precipitated by incubation of the samples for 10 minutes at 0 °C in 2.5 volume ethanol. After centrifugation the pellets were washed once in 80% ethanol and once in 99% ethanol, air-dried and resuspended in TE. The deletion breakpoints were determined by agarose gel electrophoresis of a suitable restriction digestion product of plasmid DNA. This analysis made it possible to decide which clones were to be characterized additionally by sequencing.

Sequencing

Selected clones from the preparations described in the foregoing were sequenced by the chain termination method using double-stranded, supercoiled plasmid DNA, cf. Chen E. Y. and P. H. Seeburg "Supercoil sequencing: a fast and simple method for sequencing plasmid DNA". DNA 4:165-170 (1985). Most of the clones from each aliquot had deletion breakpoints in a region of 50 to 100 base pairs. The distance between the fractions was roughly 100 to 200 base pairs.

Statistical judgement

The difference in the frequency of the individual fragments between control and samples was judged by the Fisher exact test or the chi square test with Yate's correction. The level of significance was accepted as being p less than 0.05 after correction of the p-value for the number of variable fragments observed between various individuals. The relative risk (RR) was calculated from the formula

$$RR = \frac{\text{pos. patients x neg. control individuals}}{\text{neg. patients x pos. control individuals}}$$

and the absolute relative risk (ARR) from

$$ARR = \frac{\text{pos. patients x tot. control individuals}}{\text{neg. patients x tot. control individuals}} \times \text{incidence of IDDM}$$

The incidence of IDDM was 0.38%, cf. Christian, B., H. Kromann, M. Christy, O. O. Andersen and J. Nerup. "Incidence of insulin-dependent diabetes mellitus (0-29 years at onset) in Denmark". Acta Med. Scand. Suppl. 624:54-60 (1979).

RESULTS

Sequence of the polymorphic BamHI 3.7 kb fragment

The entire nucleotide sequence of the cloned 3.7 kb fragment from an HLA-DR4 containing chromosome showed the fragment which is referred as DQβ 3.7, as being 3558 base pairs in length. When comparing this sequence to the previously published HLA-DR and DQβ-chain genes, it was possible to identify two coding regions which were almost identical with the cosII-102 HLA-DQ β-chain gene, see Proc. Natl. Acad. Sci. USA, vol. 80 pp 7313-7317 (1983), Immunology. The coding regions of the first and the second regions showed 88-98% homology with other HLA-DQ β-chain genes. The first 154 bp of the first intron (IVS 1) and the 225 bp from the third one (ISV 3) are also rather comparable (91-96%) with cosII-102 and DC3β HLA-DQ β-chain genes. All four splicing joints were in accordance with the GT/AG rule.

The predicted amino acid sequence in the second exon, which codes for part of the N-terminal end and the β1 region of the protein, just showed four amino acids in the positions 13, 26, 45 and 57 as being different from cosII-102, while there were 11 amino acid substitutions

compared with the other HLA-DR4 associated DQ β-chain sequence derived from the KT3c21 cDNA clone, see Proc. Natl. Acad. Sci. USA, Vol. 83, pp. 264-2646, Immunology. It is observed that the amino substitutions in various HLA-DQ β-chains primarily take place in the positions 25-60 and 65-80. In contrast, only two amino acid substitutions were found between DQ β 3.7 and cosII-102 in the second region. It was therefore concluded that the BamHI 3.7 fragment represents an HLA-DQ β-chain gene, and that two BamHI sites, defining the fragment, were localized in the first and the third intermediate sequences.

The IVS1 probe detects differences between HLA-DR3/4 healthy individuals and IDDM individuals

A site specific probe was constructed from the cloned DQ β 3.5 fragment. The short (166 bp) IVS1 probe was expected to hybridize to closely related DQ β-chain genes. Since the IVS1 probe moreover primarily represents non-coding sequences, which may be less preserved than their coding counterparts, a smaller degree of cross hybridization to alleles of other genes was assumed. First, 30 HLA-DR 3/4 positive Danish individuals were tested, 13 of whom being IDDM patients and 17 healthy control individuals, to find a single hybridization pattern with just 5 fragments, viz. 12, 10, 4, 3.7 and 3.2 kb. The 10 kb fragment was present in all individuals, and all (13/13) IDDM patients had the 12 kb fragment compared with 10/17 (59%) of the control individuals (p less than 0.02). Actually, all IDDM patients were 12 and 4 kb positive, while 4 different restriction enzyme patterns were found among the control individuals.

Connection between IDDM and IVS1 probe restriction fragments in a population study

The simplified restriction pattern made it possible to

test whether the IVS1 probe would reveal differences between randomly chosen IDDM patients and healthy individuals. Such a study would allow an analysis of the strength of the possible connection between IDDM and the HLA-DQ site. It was also important to find whether the ISV1 probe would reveal a single hybridization pattern in the background population to enable studies of the relative risk of developing diabetes without prior knowledge of HLA types. Accordingly, 177 healthy adult blood donors (control individuals) were compared with 113 IDDM patients.

The presence of distinct hybridization signals gave a single non-polymorphic fragment of 10 kb and just 6 variable fragments in all individuals. Among the 133 IDDM patients who had the 10 kb fragment, 25 (21%), 76 (65%) (p less than 0.001) and 16 (14%) (p less than 0.001) 2.3 or 4 restriction fragments compared with 25 (14%), 79 (45%) and (40%) among the control individuals. Only two control individuals have as much as 5 and 6 fragments, respectively. Analysis of the frequency of individual fragments among IDDM patients and control individuals revealed further differences. By correcting the p-values for the number of observed variable fragments, it was shown that 12 kb (p less than 10^{-4}) and 4 kb (p less than 10^{-4}) fragments were increased among the IDDM patients, while the 7.5 kb (p less than 10^{-4}) and 3.7 kp (p less than 10^{3}) fragments were reduced. As many as 108-113 (92% of the IDDM patients) had the 12 kb fragment and/or the 4 kb fragment compared with 112/117 (63%) of the control individuals (p less than 10^{-4}). The simultaneous presence of the 12 and 4 kb fragments was found in 40/113 (34%) IDDM patients compared with 17/117 (10%) control individuals (p less than 10^{-4}). There were no differences between sexes in the distribution frequency for various fragments. The frequency of various fragments in relation to age for onset of possible disease was analysed. Among the 43 IDDM patients with an onset age below 20, 42 (98%)

were 12 kb and/or 4 kb positive compared with 112/177 (63%) of the control individuals (p less than 10^{-4}). The 12 kb - 4 kb positive individuals amounted to 24 (56%), while only 17/177 (10%) control individuals were found (p less than 10^{-4}).

The relative risk of a young 12 kb - 4 kb positive individual of developing IDDM accordingly constitutes 91.8% compared with the control individuals who are negative for both of these fragments. The absolute relative risk was 2.2%.

The presence or absence of 12 kb and/or 4 kb fragments was also analysed in a group of 46 patients having NIDDM. Neither individual fragments nor the fragment combinations of 12 kb and 4 kb differed in frequency between NIDDM and the control individuals. It was observed that the absence of both the 12 kb and the 4 kp fragments was 7/70 (10%) among the IDDM patients having an onset age of more than 20 years compared with 17/46 (37%) of NIDDM patients (p less than 10^{-4}).

This has been found in a study of a family having an IDDM proband who were HLA-DR 3/4 and 12 kb positive. The HLA-DR type alone was not informative because the mother was DR3/4 and IVS1 12, 7.5 and 3.0 kb positive and the father DR3 and IVS1 4 kb positive. The analysis of their 5 children, however, showed that the father's DR3 was associated with a 4 kb fragment on both chromosomes, while the mother's DR3 containing chromosome had the IVS1 fragments 7.5 and 3.0 kb. The analysis shows that these two fragments had a reduced frequency among the IDDM patients. The IVS1 probe is therefore seen to cleave the HLA-DR3 haplotype into subtypes having greater or smaller probability of developing IDDM. Moreover, this family study identified an IDDM patient who is HLA-DR-/7 positive, but who was shown to have the BamHI IVS1 probe 4 kb fragment asso-

ciated with a blank HLA-DR allele.

EXAMPLE 2

Example 1 is repeated, except that the probe is labelled with Biotin instead of ^{32}P. After hybridization, the biotin is coupled to peroxidase storeptavidin, and then the probe complex is detected by reaction with 2,2' Azino-di-(3-ethyl-benzothiazoline sulfonate) which is a peroxidase substrate.

EXAMPLE 3

Cells from an individual are lysed. The DNA is isolated and applied to a membrane filter. This filter is hybridized with a synthetic DNA probe consisting of a sequence of 12 bases or more, which occur in the probe in question. The synthetic probe is labelled prior to hybridization, as described in example 1 or example 2, and detection takes place autoradiographically or enzymatically.

EXAMPLE 4

Example 3 is repeated, except that the probe is one having a sequence of least 15 bases showing 80% homology or more to a sequence of at least 15 bases in the sequence in question.

EXAMPLE 5

Example 1 is repeated, except that the probe is coupled to an arbitrary DNA sequence prior to hybridization.

Patent Claims:

1. An agent for detection of gene structures which are characteristic of humans having a tendency to develop insulin-dependent diabetes mellitus (IDDM), consisting of or containing a DNA sequence from the HLA-DQ β-chain gene, characterized in that the DNA sequence comprises 154 bp from intron 1 (IVS1) and the adjoining first 12 bp of exon 2 or reactive fragment thereof.

2. An agent according to claim 1, characterized in that it comprises part of the following DNA sequence:

GGATCCCAGGTCTGCAGCGCGAGGCACGGGCCGGCGGGAACTGGAGGTCGCGCGGG
CGGTTCCACAGCTCCGGGCCGGGTCAGGGCGGCGGCTGCGGGGTGGCCGGGCTGGG
GCCGGGCCGGGGCCGGACTGACCGGCCGGTGATTCCCCGCAGAGGATTTCGTGT

3. A process for identification of DNA structures from humans having a great tendency to develop IDDM, characterized by hybridizing chromosomes from a human cell with the agent of claim 1 or 2 in a labelled state, and then detecting the hybrid thus labelled.

4. A process according to claim 3, characterized in that the agent is a probe labelled ^{32}P, the detection being performed by autoradiography.

5. A process according to claim 3 or 4, characterized by using chromosomes from nucleated blood cells.

6. A process according to any of claims 3-5, characterized by cleaving the chromosomes with the restriction enzyme BamHI, following which the resulting

DNA fractions are subjected to gel electrophoresis, transferred to membrane and hybridized with the labelled probe.

7. A process according to any of claims 3-6, c h a r a c t e r i z e d by using a DNA sequence having at least 15 bases showing 80% homology or more to a sequence of at least 15 bases in the original sequence.

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK87/00125

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC [4]

C 12 Q 1/68

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC 4	C 12 Q 1/00, /68; G 01 N 33/53, /58
US Cl	435: 5, 6

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

SE, NO, DK, FI classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Chemical Abstracts, Vol 104, 1986, abstract 46600q, Proc Natl Acad Sci U S A 1985, 82(23), 8139-43	1
Y	Chemical Abstracts, Vol 105, 1986, abstract 147440p, Hum Immunol 1986, 17(1), 61-8 (Eng)	1, 3-6
Y	WO, A1, 83/03260 (PETERSON P A ET AL) 29 September 1983	1, 3-6
Y	MEDLINE, NLM Accession No 86 253068, J Exp Med 1986 Jul 1;164(1):345-50	1, 3-6
Y	MEDLINE, NLM Accession No 86 202269, Lancet 1986 May 10;1(8489):1058-60	1, 3-6
E	WO, A1; 86/07464 (GENETIC SYSTEMS CORPORATION) 18 December 1986	1, 3-6
E	EP, A1, 0 237 362 (CETUS CORPORATION) 16 September 1987 see p 33-41 .../...	1

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1988-01-18	1988 -01- 25
International Searching Authority	**Signature of Authorized Officer**
Swedish Patent Office	Carl Olof Gustafsson

III. DOCUMENTS CONSIDERED TO BE RELEVANT (C NTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	EP, A2, 0 084 796 (CETUS CORPORATION) 3 August 1983	1,3
A	US, A, 4 623 619 (OWERBACH ET AL) 18 November 1986	1,2
A	NATURE, Vol 322, 64-67, published 3 July 1986, (H FESTENSTEIN ET AL) "New HLA DNA polymorphisms associated with autoimmune diseases"	1, 3-6
Y	Biomed Biochim Acta Vol 44, 33-36, published 1985 no 1, (B MICHELSEN) "Indentification of an HLA-DQ β-chain related genomic sequence associated with insulin-dependent diabetes"	1, 3-6
P	Biotechnology Vol 4, 975-981, published November 1986 (H A ERLICH ET AL) "HLA Typing Using DNA Probes" see in particular fig 3B and fig 5	1, 3-6